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Effectiveness of Copper-Oxide Wire Particles on the control of Haemonchus contortus in sheep

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EFFECTIVENESS OF COPPER-OXIDE WIRE PARTICLES ON THE CONTROL OF *HAEMONCHUS CONTORTUS* IN SHEEP

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DEDICATION

Mrs. Diane Williams Watkins

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ABSTRACT

Among the gastrointestinal nematode parasites that cause the most problems to small ruminants, *Haemonchus contortus* is one of major concern. Currently, the control of *H. contortus* and others is almost entirely based on the use of anthelmintics. Consequently, anthelmintic resistance has developed worldwide and this has become a serious problem in small ruminant nematode parasite control programs. In view of this, there is a need for alternative control methods. The use of Copper-Oxide Wire Particles (COWP) to help reduce parasite burden is one such alternative. Three trials were conducted to determine the effect of COWP on the reduction of *H. contortus* in ewes (Summer, 2002, and Spring, 2003) and lambs (Summer, 2002). Each trial followed similar protocols where the animals were allocated to treatment and control groups based on fecal egg count (FEC). COWP boluses were administered to the treatment group and infection level was monitored over a period of time by weekly determination of FEC and blood PCV. Serum copper levels were determined before and at the end of each trial. Feces were also collected every other week for coproculture, which was used to determine relative distribution of infective larvae genera. Results of all three trials indicated that COWP were effective in reducing FEC for a period of 4-5 weeks. There was no difference in PCV between groups for any trial. Coproculture indicated that the reduced FEC was primarily due to a reduction in *H. contortus*. Serum copper levels were either below or within normal range before treatment and remained within normal limits at the end of the trials. The results from these trials demonstrated that the use of COWP reduced *H. contortus* infection and this may be useful in conjunction with other nematode parasite control methods.

CHAPTER ONE

INTRODUCTION

Annually in the United States, an estimated loss of 270 million dollars in sheep and goat production is attributed to parasites (Baker et al., 1990). It has been calculated that internal parasitism, largely by trichostrongylid nematodes, causes losses equivalent to 19 percent of the sheep industry (Barriga, 1997). Stomach/intestinal worms have been and still are the number one concern of sheep producers. This has been confirmed by three USDA sheep surveys. The top health condition of moderate or high concern to U.S. sheep producers was stomach/intestinal worms (62.1 percent of operations) (USDA, 1996a). The East South Central region (Alabama, Arkansas, Kentucky, Louisiana, Mississippi, and Tennessee) contained the highest percentage (77.8) of sheep producers with this concern (USDA, 1996b). In the five years prior to the 1996 survey, stomach/intestinal worms was still the top health condition known to be present (suspected or confirmed) on sheep operations (48.6 percent) (USDA, 1996b). In the most recent survey, the most common disease present (suspected or confirmed) in flocks within the three previous years was stomach/intestinal worms (74.0 percent) (USDA, 2003).

The trichostrongylids are the most harmful nematode parasites of small ruminants. Within the Superfamily Trichostrongyloidea, several species contribute to parasitism in sheep (Barriga, 1997). The four of major concern are *Haemonchus*, *Trichostrongylus*, *Cooperia*, and *Oesophagostum*. Of these four, the number one parasite that causes the most problems to small ruminants is *Haemonchus contortus*.

Haemonchus contortus is a major cause of ill health and economic loss in sheep production. *H. contortus* is commonly called the "barber pole" worm because of the physical features of the female. The female's white, egg filled uterus is wound in a helix around the blood-filled gut, which looks like a barber pole. This nematode is a blood-sucking parasite that pierces the lining of the abomasum, causing blood plasma and protein loss to the host. The pathogenic effects of *H. contortus* result from the inability of the host to compensate for the blood loss (Bowman, 1995). At peak infection, naturally acquired populations of *H. contortus* may remove one fifth of the circulating erythrocyte volume per day from lambs and may remove an average of one tenth of the circulating erythrocyte volume per day over the course of nonfatal infections lasting two months (Bowman et al., 2003).

The symptom most commonly associated with *H. contortus* infection is anemia. Anemia is characterized by pale mucous membranes, especially in the lower eyelid. Lambs are often the most seriously affected members of a flock, but older sheep under stress also may have fatal anemia (Bowman et al., 2003). Edema (bottle jaw) is also a sign of infection and is an accumulation of fluid under the jaw. (Barriga, 1997).

The greatest economic effects of *H. contortus* are found in warm humid areas of the world. In addition to losses through mortality (especially of pastured lambs in mid-to-late summer), major losses are attributed to reduced feed efficiency, slow rate of gain, poor reproductive efficiency, lowered production of wool and meat, and labor and drugs associated with control. (Hartwig, 2000). Due to the economic effect this nematode parasite and others have on the host, it is necessary to have adequate methods of control.

Currently, the control of nematode parasites is almost entirely based on the use of anthelmintics. Due to the overuse and or misuse of this previously effective control measure, anthelmintic resistance has occurred. Anthelmintic resistance is when the drug is no longer effective at recommended dosages. The development of anthelmintic resistance has dramatically increased worldwide. This resistance has mostly been found in small ruminants and horses. In sheep, anthelmintic resistance appeared in geographical regions where *H. contortus* predominates and where the annual numbers of cycles of infection and anthelmintic treatments are numerous (Urquhart et al., 1996).

Since the main method of controlling *H. contortus* has become relatively ineffective, other methods need to be found. There are many alternative methods of control currently being researched, but this paper will focus on the use of copper-oxide wire particles (COWP). After being introduced to the rumen via gelatin capsules, the COWP are released and pass directly to the abomasum, where they adhere to the mucosa and release free copper, resulting in constant elevation of soluble copper (Bang et al. 1990a). The activity of COWP is reputed to be related to the soluble copper concentration in the digestive tract (Chartier et al., 2000). This concentration of copper creates an environment that somehow affects the nematodes ability to remain established. Thus, they are expelled.

The objective of this project was to determine the effect of COWP on the reduction of *H. contortus* in growing and mature sheep.

CHAPTER TWO

LITERATURE REVIEW

2.1. Ecology of Nematode Parasites

The species of nematodes that affect sheep the most belong to the Superfamily Trichostrongyloidea and include *Haemonchus*, *Trichostrongylus*, *Cooperia*, *Ostertagia*, and *Oesophagostomum* (Bowman et al., 2003). Each of these nematodes goes through a direct life cycle, meaning that no intermediated host is needed for the life cycle to be completed.

The females of *H. contortus* are prolific egg layers (Urquhart et al., 1996). Passage of eggs begins between days sixteen to twenty-three of infection for most genera (Barriga, 1997). Once on pasture, the eggs hatch and depending on the environmental conditions develop (molt) from the free-living first stage larvae (L1), to the free-living second stage larvae (L2) and the finally to the free-living infective third stage larvae (L3) which retains the cuticle of the L2 (Urquhart et al., 1996; Barriga, 1997). This can happen (egg hatching to the L3) in as short of a period as five days, but development may be delayed for weeks or months under cool conditions (Urquhart et al., 1996).

The L3 migrate from the feces onto the grass when a moisture medium is present (rain, flood, heavy dew). Once on the grass, they are ingested by grazing sheep. Inside of the host, the sheath of the L3 is cast off in the rumen and then exsheated L3s move to the abomasum where they penetrate the mucosa. By day three to seven in the mucosa they undergo a molt to the fourth stage larvae (L4) (Bowman et al., 2003). The L4s then return to the lumen to lacerate the mucosa and ingest the seeping blood (Barriga, 1997).

After a variable period of time (usually by day ten to fifteen, if arrested development hasn't occurred) the L4 mature and become adults, completing the cycle (Hartwig, 2000). As egg producing adults, the cycle continues. Until recently, strategic use of effective anthelmintics and pasture management has been able to break this cycle.

2.2. Anthelmintic Use and Resistance

Anthelmintics are the most widely used means to control helminth infections (Roos, 1997). The control of nematode parasites is essential for maximizing livestock productivity and feed efficiency. However the control of nematode parasites is becoming more difficult with the increasing resistance of the parasites to common anthelmintics. (Ketzis, 2003). The frequent use of anthlemintics can select a subpopulation of parasites that are resistant to the mode of action at the concentration used. With time, this subpopulation may overwhelm and replace the previously susceptible population (Barriga, 1997).

The earliest reports of anthelmintic resistance involved nematode parasites of sheep and horses. Now resistance has appeared in parasites that affect many animal industries as well as humans; which includes several phyla of helminths and covers all of the major chemical groups of anthelmintics (Sangster and Gill, 1999). Parasite resistance to benzimidazoles (i.e. albendazole, thiabendazole, fenbendazole), imidazothiazoles (i.e. levamisole), and macrolides (i.e. ivermectin) has been reported in Africa, Australia, Europe, North America and South America; wherever animals are regularly treated with anthelmintics and investigations have been made (Prichard, 1994). Unfortunately, irreversible resistance develops in helminths, usually within five years of the introduction

of the anthelmintic. Resistance of parasitic helminthes to anthelmintics is becoming a serious problem in Veterinary Medicine, especially in sheep husbandry (Roos, 1997).

Because chemical control is the backbone of parasite control, resistance is its inseparable consequence and every effort must be made to delay the onset of resistance. The main methods for delaying drug resistance are: infrequent use of anthelmintics, utilization of the most active anthelmintic compounds at the highest practical dose, yearly alternation of anthelmintics from different groups, management of pastures to avoid the buildup of resistant populations, and surveillance of newly acquired stock (Barriga, 1997).

Delaying the onset of resistance thru the above mention means are reasonable and practical to help control nematodes, but it is necessary to move away from anthelmintic use as the primary method and look at other methods.

2.3. Alternative Parasite Control

As a consequence of anthelmintic resistance, considerable research effort has been expended on alternative approaches to the control of nematodes of livestock. According to Ketzis (2003) some of these include: nematode-trapping fungi and feed additives that increase the population of fungi; pasture plants that decrease parasite-larvae populations on pastures; forages and medicinal plants that decrease adult parasite populations in the host; and dietary changes (i.e. COWP) that increase the host's immunological response to parasite infections and decrease production losses caused by parasites. Other control methods being researched are breeding of resistant or resilient hosts (Waller, 1997, 1999); use of helminth vaccines (Emery et al., 1993; Waller, 1999); the use of copper in various forms (Waller, 1999; Bang et al., 1990a; Knox, 2002; Nyman, 2000; Chartier et

al., 2000), and diatomaceous earth which is reported to lacerates the cuticle of the nematode which results in dehydration and death.

Of these methods, the two that seem to be accepted as having the most short-term potential to achieve to the best results are nematode-trapping fungi and COWP. Work on the use of fungi to control livestock parasites dates back to 1939 (Waller and Larsen, 1993). Experiments using *Duddingtonia flagrans* have been among the most successful and this fungus has reduced the percentage of L3 on pasture by 76.6 to 100 percent (Pena et al., 2002). As for COWP, Bang et al. (1990b) reported that there was an interaction between copper metabolism and gastrointestinal nematodes. Also, Bang et al. (1990a) demonstrated the anthelmintic activity of COWP against nematodes in experimentally infected sheep.

2.4. Copper-oxide Wire Particles

The forms of copper most commonly used in animal feed are copper sulfate, copper oxide, copper carbonate, and tribasic copper chloride. Copper oxide is manufactured by roasting copper metal in an oxidizing furnace or by solubilizing copper metal with acid and treating it with a caustic to precipitate copper oxide. (Prince Agri Products, 2003). Copper is a necessary trace element in the diet. Maximum immune response is dependent on copper as indicted by depressed antibody titers in deficient animals (Salt Institute, 2002).

Because of the effect that copper has on the body (man and animal) COWP have been used for many years to treat copper deficiency (Suttle, 1981; Judson et al., 1982, 1984; Langlands et al., 1983; Dewey, 1997). But COWP are not only an efficient and effective means of treating copper deficiency in grazing livestock, it can also be

potentially useful as an anthelmintic (Dewey, 1977). After dosing, COWP flow with ingesta from the rumen and lodge in the folds of the sheep's abomasum where the low pH induces the release of high concentrations of soluble copper, which have an adverse affect against abomasal species of nematodes (Knox, 2002). Because of the rapid increase in anthelmintic resistance, this control method is continually being evaluated.

The reported anthelmintic effect of COWP has been seen in numerous studies (Bang et al., 1990a; Nyman, 2000; Chartier et al., 2000; Knox, 2002). In all of those studies the nematode burden of *H. contortus* was reduced by using the COWP. Each of those studies (except Nyman, 2000) involved experimental infection with *H. contortus* either once at the beginning of the study or weekly doses for the duration of the study. The objective of this study was to evaluate the effect that COWP have on reducing the nematode burden in sheep under natural grazing conditions.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Location

The location of this experiment was at the Ben Hur Research Farm Sheep Unit, Louisiana State University Agricultural Experiment Station, Baton Rouge, Louisiana.

3.2. Animals and Trial Length

All of the animals were naturally infected by grazing on pastures where *H contortus* and *Trichostrongylus spp.* are the dominate nematodes.

3.2.1. Trial 1

This trial consisted of 28 mature F1 (Suffolk X Gulf Coast Native) ewes and 20 growing Katahdin lambs. This trial was conducted over a 12 week period (June 24-September 17, 2002) for the ewes and over a 14 week period (June 24-October 1, 2002) for the lambs.

3.2.2. Trial 2

This trial consisted of 16 F1 ewes and the trial duration was over a 4 week period (November 19-December 17, 2002).

3.2.3. Trial 3

This trial consisted of 30 ewes (Suffolk, F1, and Gulf Coast Native) and the trial duration was over a 6 week period (February 18-April 1, 2003). At the beginning of the trial, the ewes were pregnant, which then lambed and nursed for the duration of the trial.

3.3. Experimental Design

For each trial, the animals were randomly allocated to treatment groups based on initial fecal egg count (FEC), which was done to insure that each group had relatively equal parasite burdens. At the start of each trial (week 0), the treated group received a gelatin

capsule bolus containing either four grams (ewes) or two grams (lambs) of COWP (Copinox, Animax Ltd). The lambs received at second COWP bolus at week 6 of Trial 1.

3.4. Techniques

3.4.1. Serum Copper

At the beginning and end of Trials 1 and 3, blood was collected from each animal by jugular venipuncture into 7-ml red top serum vacutainer tubes. Serum Cu was determined (Toxicology Laboratory, School of Veterinary Medicine, Louisiana state University) to establish pre- and post-treatment levels. Cu can be relatively toxic to sheep, so this was measured to denote any change due to the treatment.

3.4.2. Packed Cell Volume

For the duration of each of Trials 1 and 3, blood samples were collected weekly by jugular venipuncture into 7-ml purple top EDTA vacutainer tubes. These samples were analyzed for packed cell volume (PCV) levels. Micro-hematocrit capillary tubes were filled with whole blood, sealed, and centrifuged in an Autocrit centrifuge for five minutes. The micro-hematocrit for each animal was read directly from the centrifuge scale. This was done to monitor the level of anemia in each animal throughout the trial period.

3.4.3. Fecal Egg Count

Fecal samples were collected directly from the rectum of all animals at weekly intervals for each trial. FEC was determined using the modified McMaster technique (Whitlock, 1948). Feces from each animal were thoroughly mixed in a saturated salt solution. A random sample of the mixture was transferred into both sides of the McMaster chamber

and the trichostrongyle-type eggs were counted. The FEC allowed us to evaluate the relative nematode burden in each animal and group.

3.4.4. Fecal Cultures

Bulk fecal cultures were performed every two weeks in each trial to allow the eggs to hatch to L3 so they could be identified to genus. The feces from the control and treated groups were mixed in separate containers except for week 0 for Trials 1 and 2 which were combined because the FEC was similar. It was inferred that the distribution of nematode genera in each would also be similar. For each culture, ten grams of thoroughly mixed feces was measured out and placed in a plastic cup with four small holes in the bottom. Additional ten gram cultures were processed until there were an equal number of replicates for each group. An approximately equal amount of vermiculite was mixed with the feces and water was added to make a crumbly wet culture. The top of the cup was covered in cheesecloth and turned upside down in a larger plastic cup. The larger cup contained approximately 5 ml of water to provide a relatively saturated/humid environment for egg development. Each culture was sealed in a plastic bag and left at room temperature for ten to fourteen days. Subsequently, the larger cup was filled with warm water to a level in which the fecal culture material was emerged. This was left standing for 4 to 6 hours to allow the L3s to migrate thru the culture material and collect in the larger cup. After this, the small cup containing the culture material was removed and the culture material was discarded. The water in the larger cups was carefully suctioned off to a volume less than 50 ml. The remaining solution was mixed thoroughly and placed in a 50 ml centrifuge tube. After no less than 3 hours, the 50 ml centrifuge tubes were suctioned down to a volume of less than 15 ml

and transferred to 15 ml centrifuge tubes where a drop or two of formalin was added to preserve the L3.

3.4.5. L3 Identification

The 15 ml centrifuge tubes obtained from the fecal cultures were suctioned off to between one and three ml. A few drops of the culture solution and a drop of iodine were placed on a microscope slide using a glass pipet. A cover slip was placed on top and the L3 were then identified to genus.

3.5. Calculations

3.5.1. Percent Reduction

The reduction for FEC (%) and the percent reduction of *H. contortus* L3 were calculated as:

$$[(\text{control mean} - \text{treated mean}) \div \text{control mean}] \times 100.$$

When results were negative, the percent reduction was considered zero.

3.5.2. Statistical Analysis

The statistical difference between COWP treated and control groups for FEC, PCV, and *H. contortus* L3 percentage in fecal cultures was analyzed in SAS using PROC TTEST. Differences were considered statistically significant when $p \leq 0.05$.

CHAPTER FOUR

RESULTS

4.1. Trial 1 Lambs

4.1.1. Fecal Egg Count

The lamb FEC showed that there was a substantial reduction in the treated group from week 1 through week 10 (Table 1, Figure 1). This reduction was the greatest from week 1 to week 4 and week 7 to week 10. These two periods correspond to the weeks immediately after COWP bolus administration. The reduction was significant for weeks 1, 2 and 4 of the trial.

Table 1. Trial 1 mean fecal egg count (FEC; eggs per gram) and percent reduction comparing copper-oxide wire particle bolus treated and control lambs.

Week	Control	Treated	FEC reduction (%)
0*	2165	2340	0
1^{+}	2765	240	91.3
2^{+}	3090	45	98.5
3	3556	180	94.9
4^{+}	1725	545	68.4
5	2795	1620	42
6*	5700	4080	28.4
7	1035	545	47.3
8	1190	490	58.8
9	3090	870	71.8
10	2900	1695	41.6
11	1175	1000	14.9
12	1255	1390	0
13	1055	1515	0
14	1330	1750	0

*2 gram copper-oxide wire particles bolus administered to the treated group.
$^{+}P \leq 0.05$

Subsequent to week 10, the effect of the COWP bolus was not evident.

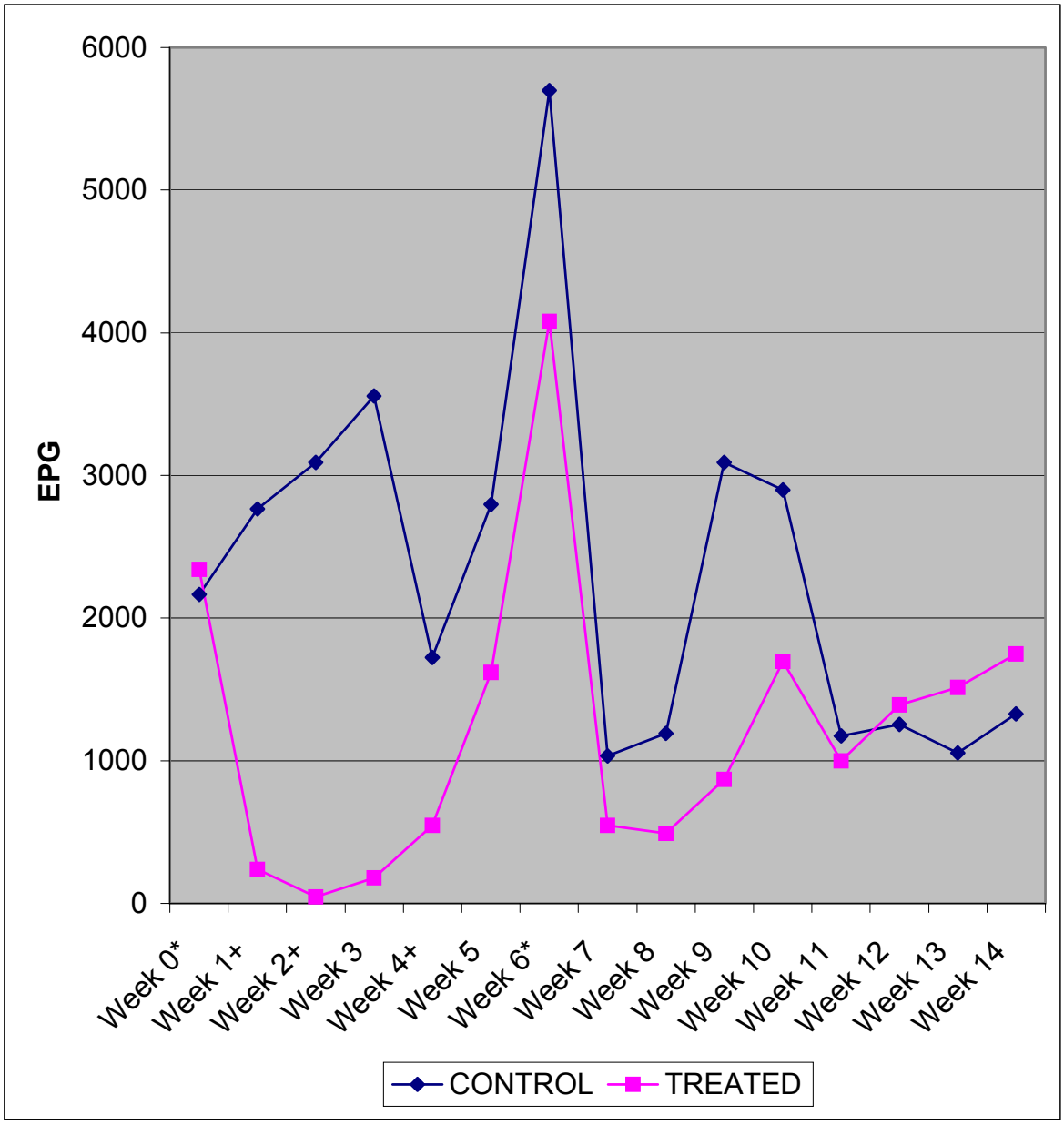

Figure 1. Trial 1 mean fecal egg count comparing copper-oxide wire particle bolus treated and control lambs. *2 gram copper-oxide wire particles bolus administered to the treated group. $^{+}P \leq 0.05$

The largest difference, measured by FEC percent reduction, was seen in weeks 1, 2, and 3, where the reduction was 91.3%, 98.5%, and 94.9%, respectively (Figure 2).

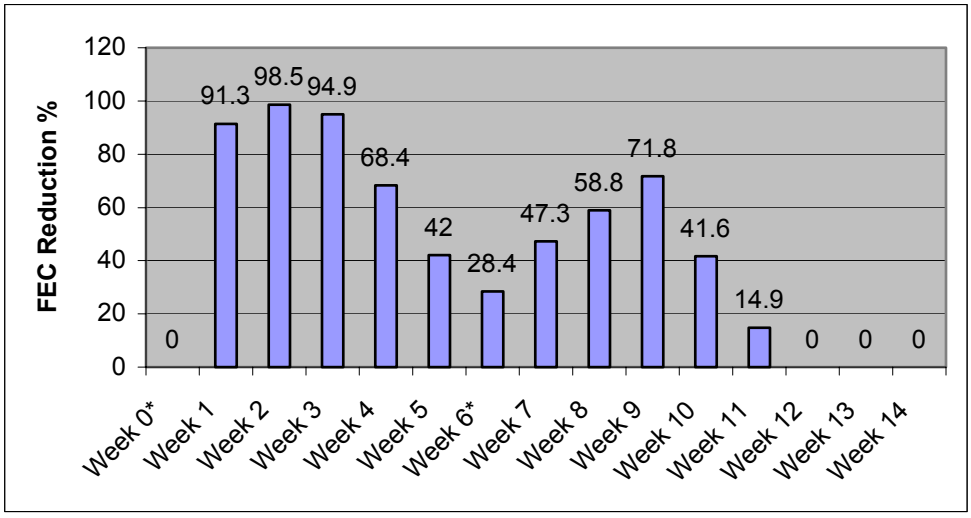

Figure 2. Trial 1 mean percent reduction in FEC comparing copper-oxide wire particle bolus treated and control lambs. *2 gram copper-oxide wire particles bolus administered to the treated group.

The highest FEC for both groups was seen in week 6 when the control group had a mean FEC of 5700 epg and the treated group had a mean FEC of 4080 epg. Due to this high infection level, a second COWP bolus was administered, and again a substantial drop in the FEC was observed in the treated group. There was also a drop in the control group FEC, but it remained higher than the treated group. After the second bolus, there was a steady rise in FEC percent reduction from week 7-9.

4.1.2. Packed Cell Volume

The PCV values for both groups were similar throughout the trial and no significant differences were observed (Figure 3).

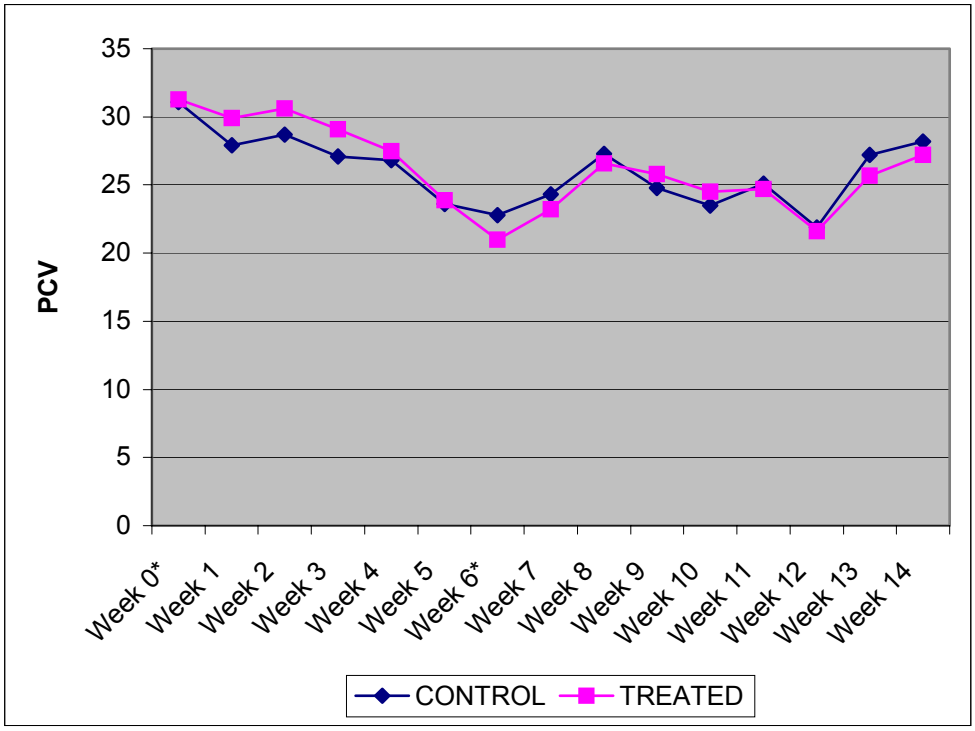

Figure 3. Trial 1 mean packed cell volume comparing copper-oxide wire particle bolus treated and control lambs. *2 gram copper-oxide wire particles bolus administered to the treated group.

Although no significant differences were observed, the treated group did have higher PCV levels for the first four weeks and weeks 9 and 10, and the control group PCV levels were higher for weeks 6-8, and 11-14. The lowest PCV level (21) in the treated group was seen at week 6. This was during the same time when nematode infection levels in both groups were at their highest (Table 1). The lowest PCV level (21.9) in the control group was seen during week 12.

4.1.3. Larval Cultures

The ability of the COWP to reduce *H. contortus* infection was measured by the percent reduction in percentage of *H. contortus* L3 (Table 2, Figure 4).

Table 2. Trial 1 *Haemonchus contortus* L3 percentage and percent reduction comparing copper-oxide wire particle bolus treated and control lambs.

Week	0*	2+	4+	6	8+	10+	12	14+
Control	92.3	92.3	92.4	90.7	66.4	74.8	52.8	45.6
Treated		35	44.8	87.8	14.5	52.8	44	25.7
Percent reduction	0	62.1	51.5	3.2	78.2	29.4	16.7	43.6

*2 gram copper-oxide wire particles bolus administered to the treated group.
$^{+}P \leq 0.05$

The reduction in *H. contortus* was highest during the few weeks subsequent to each of the COWP bolus administrations. The treated group percent reduction was significantly lower than the control group for weeks 2, 4, 8, 10 and 14.

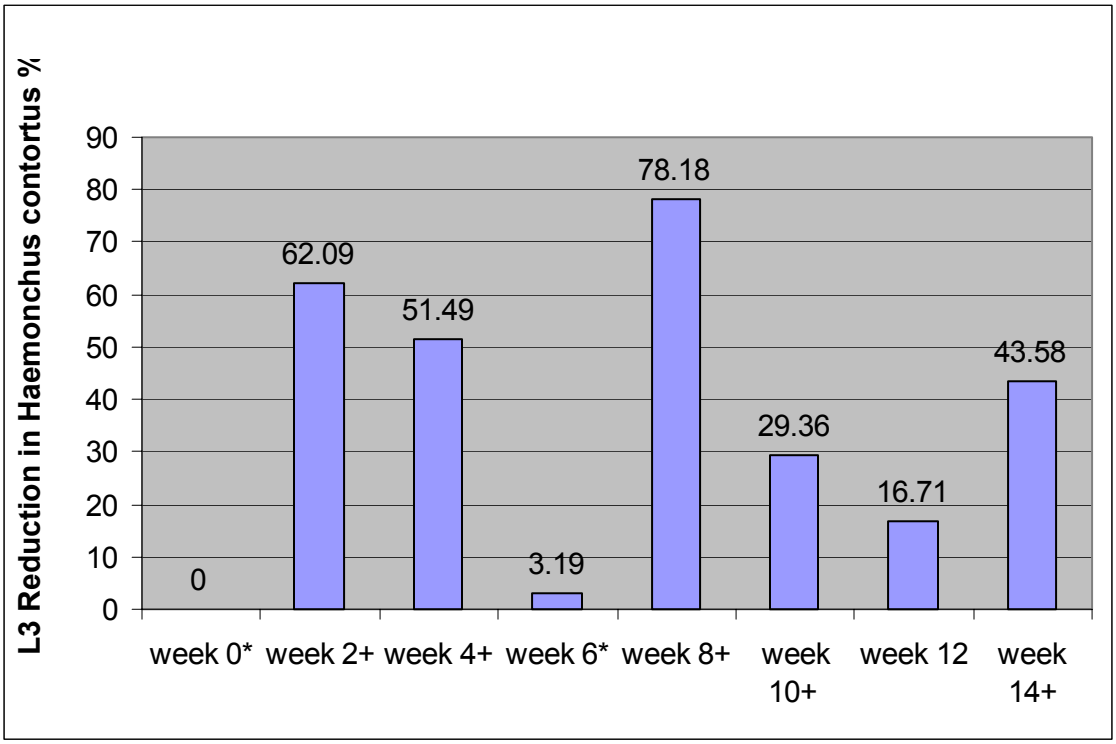

Figure 4. Trial 1 *Haemonchus contortus* L3 percent reduction comparing copper-oxide wire particle bolus treated and control lambs. *2 gram copper-oxide wire particles bolus administered to the treated group. $^{+}P \leq 0.05$

The percent reduction in *H. contortus* was lowest at the start of the study and week 6. The percent reduction in *H. contortus* was considered substantial (≥ 50 percent) during weeks 2, 4, and 8.

4.1.4. Serum Copper

The mean serum copper levels in both groups were similar pre- and post-treatment (Table 3).

Table 3. Trial 1 pre- and post- treatment serum copper levels (ppm) per animal in control and treated lambs.

Animal ID	Group	Pre	Post	Difference	Comment
2278	Control	1.1	0.3	-0.8	Lower
2281	Control	0.7	0.8	0.1	Higher
2283	Control	0.6	0.6	0	Same
2284	Control	0.8	0.5	-0.3	Lower
2285	Control	0.7	0.5	-0.2	Lower
2287	Control	0.7	0.7	0	Same
2290	Control	0.6	0.4	-0.2	Lower
2293	Control	0.5	0.5	0	Same
2296	Control	0.7	0.4	-0.3	Lower
2299	Control	0.7	0.5	-0.2	Lower
Mean Cu	Control	0.71	0.52	-0.19	Lower
2277	Treated	1.1	0.7	-0.4	Lower
2279	Treated	0.7	0.5	-0.2	Lower
2282	Treated	0.6	0.7	0.1	Higher
2286	Treated	0.7	0.4	-0.3	Lower
2288	Treated	0.8	0.8	0	Same
2289	Treated	1	0.7	-0.3	Lower
2292	Treated	0.6	0.5	-0.1	Lower
2295	Treated	0.6	0.4	-0.2	Lower
2298	Treated	0.7	0.5	-0.2	Lower
2300	Treated	0.6	0.6	0	Same
Mean Cu	Treated	0.74	0.58	-0.16	Lower

At the start of the trial, the majority of the animals had serum Cu levels within the normal range of 0.7 to 1.4 ppm. At the end of the trial all but two of the animals' serum Cu levels were lower.

4.2. Trial 1 Ewes

4.2.1. Fecal Egg Count

Results for this trial were atypical (Table 4, Figure 5).

Table 4. Trial 1 mean fecal egg count and percent reduction comparing copper-oxide wire particle bolus treated and control ewes.

Week	0*	1^{+}	2	3	4	5	6	7	8	9	10	11	12
Control	2779	3386	32	4	75	93	143	300	625	707	475	89	150
Treated	2814	136	0	4	75	150	111	154	421	161	268	464	157
FEC reduction (%)	0	96	100	0	0	0	23	49	33	77	44	0	0

*4gram copper-oxide wire particles bolus administered to the treated group. $^{+}P \leq 0.05$

The FEC was similar between groups at week 0. There was a substantial and significant drop (96%) at week 1 and subsequently (week 2) the control group FEC dropped to the treatment group level. Both groups remained relatively similar for the rest of the trial.

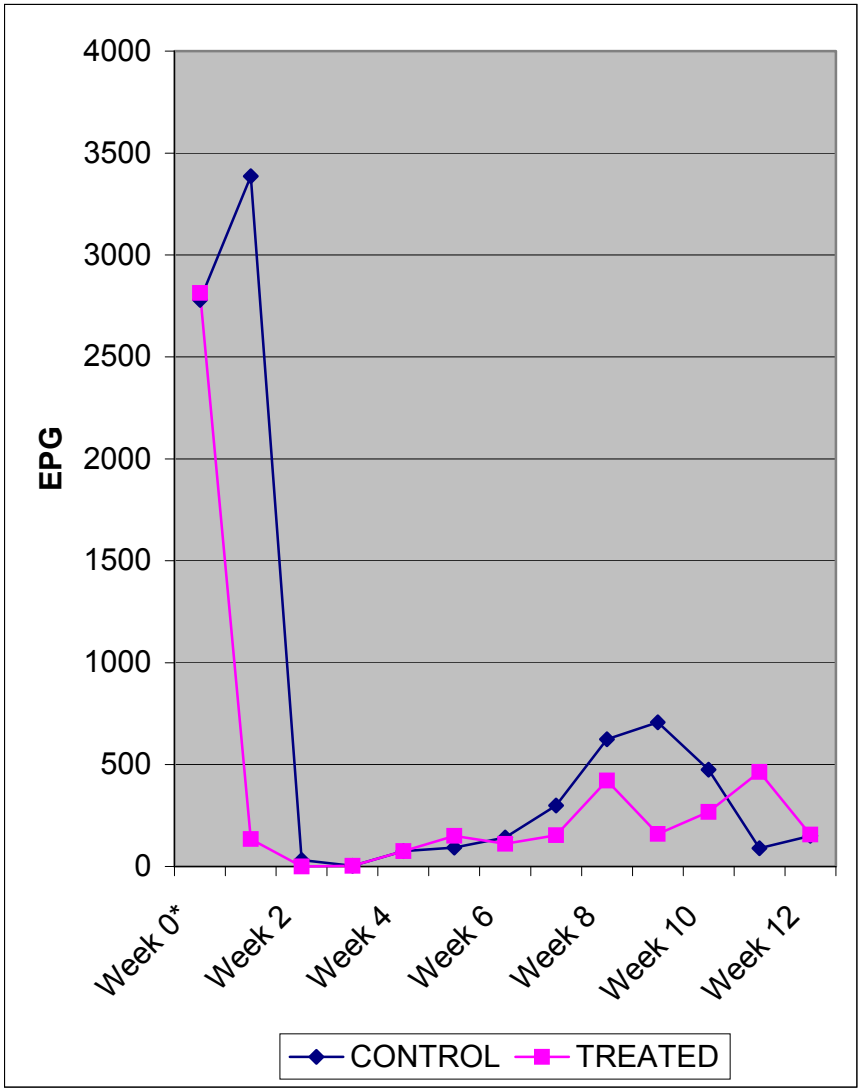

Figure 5. Trial 1 mean fecal egg count comparing copper-oxide wire particle bolus treated and control ewes. * 4gram copper-oxide wire particles bolus administered to the treated group. $^{+}P \leq 0.05$.

Week 1 was the only time that an obvious and significant difference was observed between the two groups as measured by percent reduction (Figure 6). Subsequent to week 1, infection levels were too low to derive any meaningful inferences.

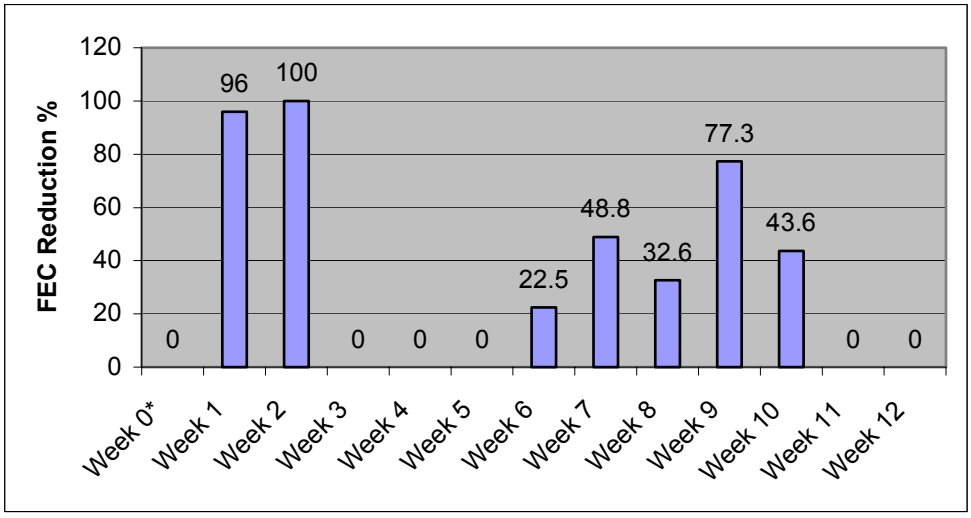

Figure 6. Trial 1 mean percent reduction in FEC comparing copper-oxide wire particle bolus treated and control ewes. *4 gram copper-oxide wire particles bolus administered to the treated group.

4.2.2. Packed Cell Volume

The PCV levels for the ewes were relatively similar throughout the trial; however, the treated groups' PCV level remained slightly higher than the control group for each week (Figure 7). No significant difference between the COWP bolus treated and control ewes were observed in this trial.

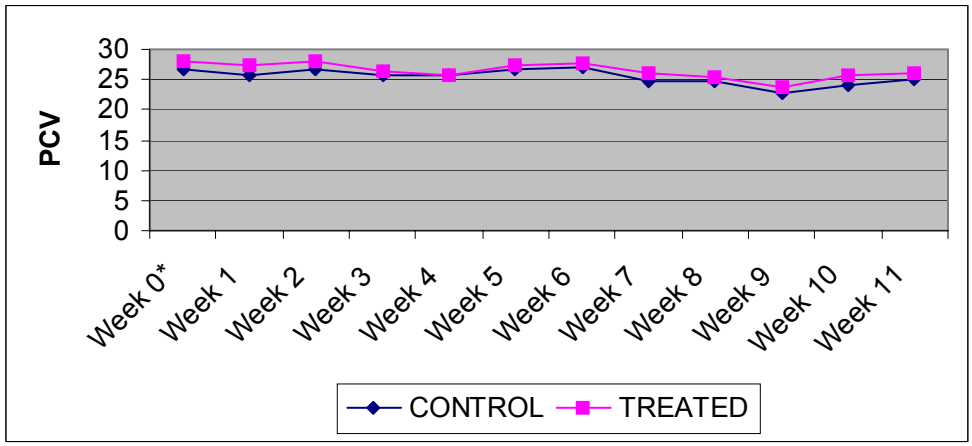

Figure 7. Trial 1 mean packed cell volume comparing copper-oxide wire particle bolus treated and control ewes. *4 gram copper-oxide wire particles bolus administered to the treated group.

4.2.3. Larval Cultures

Because of the low infection level subsequent to week 1 for this trial, larval cultures were only done for weeks 0, 8, 10, and 12 when FEC were high enough to warrant culturing (Table 5, Figure 8).

Table 5. Trial 1 *Haemonchus contortus* L3 percentage and percent reduction comparing copper-oxide wire particle bolus treated and control ewes.

Week	0*	8	10	12
Control	100	79.3	68.8	59.6
Treated		79.1	57	66.3
Percent reduction	0	0.1	17.2	0

* 4 gram copper-oxide wire particles bolus administered to the treated group.

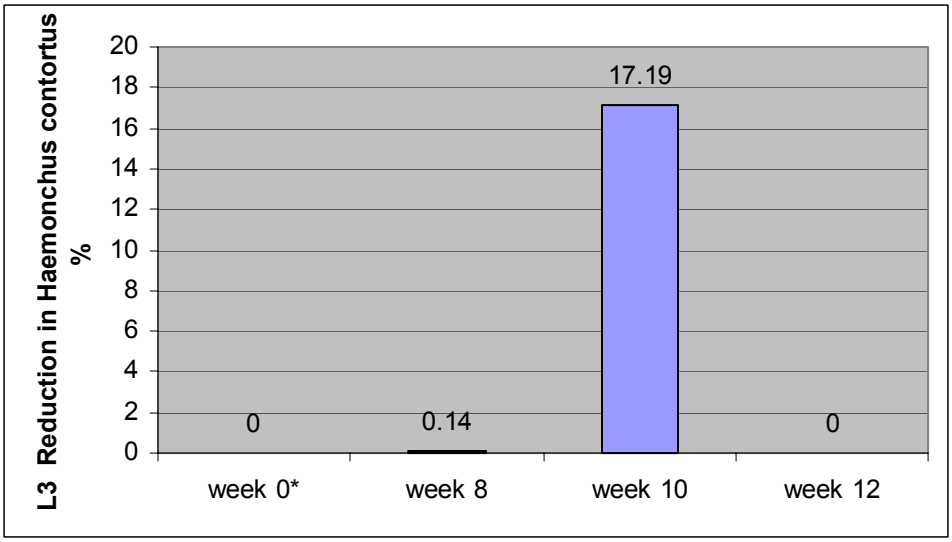

Figure 8. Trial 1 *Haemonchus contortus* L3 percent reduction comparing copper-oxide wire particle bolus treated and control ewes. *4 gram copper-oxide wire particles bolus administered to the treated group.

Due to the similar results observed, there was no reduction in *H. contortus* and no significant difference between the treated and control ewes at these time points.

4.2.4. Serum Copper

The mean serum copper levels in both groups were relatively similar at pre- and post-treatment (Table 6).

Table 6. Trial 1 pre- and post- treatment serum copper levels (ppm) per animal in control and treated ewes.

Animal ID	Group	Pre	Post	Difference	Comment
6561	Control	0.6	0.2	-0.4	Lower
6582	Control	0.6	0.9	0.3	Higher
6685	Control	0.7	0.6	-0.1	Lower
7781	Control	0.7	0.8	0.1	Higher
7804	Control	0.7	0.5	-0.2	Lower
7805	Control	0.6	0.4	-0.2	Lower
7938	Control	0.9	0.7	-0.2	Lower
8026	Control	0.6	0.4	-0.2	Lower
8050	Control	0.7	0.3	-0.4	Lower
8079	Control	0.6	0.5	-0.1	Lower
9238	Control	0.5	0.7	0.2	Higher
9260	Control	0.7	0.5	-0.2	Lower
9265	Control	0.8	0.3	-0.5	Lower
9290	Control	0.8	0.3	-0.5	Lower
Mean Cu	Control	0.678571	0.507143	-0.17143	Lower
6562	Treated	0.8	0.6	-0.2	Lower
6637	Treated	1.1	0.9	-0.2	Lower
6659	Treated	0.5	1	0.5	Higher
6689	Treated	0.5	0.4	-0.1	Lower
6692	Treated	0.6	0.6	0	Same
7730	Treated	0.8	0.5	-0.3	Lower
7787	Treated	0.7	0.5	-0.2	Lower
7847	Treated	0.7	0.4	-0.3	Lower
7864	Treated	0.8	0.6	-0.2	Lower
9219	Treated	0.7	0.5	-0.2	Lower
9247	Treated	0.7	0.5	-0.2	Lower
9271	Treated	0.7	0.6	-0.1	Lower
9282	Treated	0.8	0.5	-0.3	Lower
9291	Treated	0.7	0.3	-0.4	Lower
Mean Cu	Treated	0.721429	0.564286	-0.15714	Lower

At the start of the trial the animals' serum Cu levels were close to or at the lower limit of 0.7 ppm. At the end of the trial, the majority of animals' serum Cu levels remained at or close to the lower limit.

4.3. Trial 2 Ewes

4.3.1. Fecal Egg Count

The FEC was similar at week 0 between groups and was substantially reduced in the COWP treated ewes from week 1 through week 4, although a significant difference was not observed (Table 7, Figure 9).

Table 7. Trial 2 mean fecal egg count and percent reduction comparing copper-oxide wire particle bolus treated and control ewes.

Week	0*	1	2	3	4
Control	475	663	531	506	494
Treated	538	369	163	225	169
FEC reduction (%)	0	44	69	56	66

*4 gram copper-oxide wire particles bolus administered to the treated group.

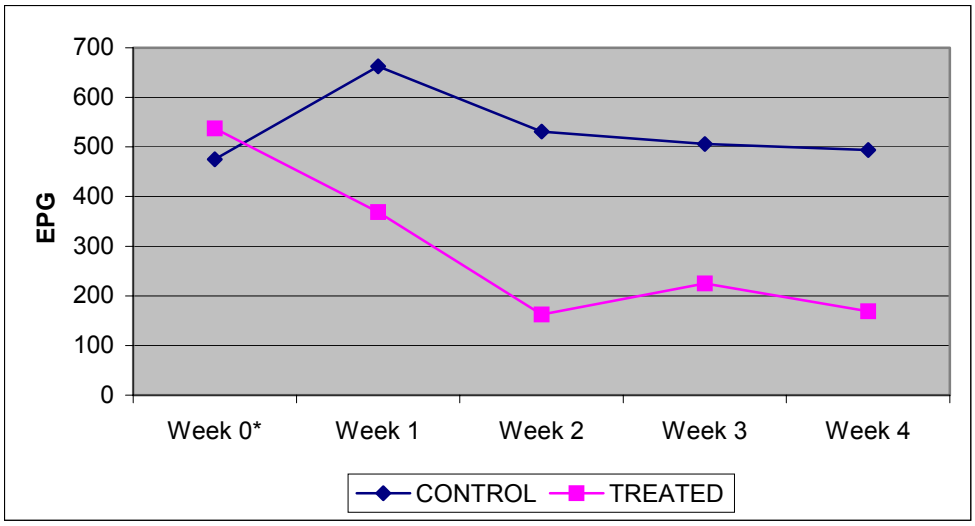

Figure 9. Trial 2 mean fecal egg count comparing copper-oxide wire particle bolus treated and control ewes. *4 gram copper-oxide wire particles bolus administered to the treated group.

The percent reduction was relatively consistent for week 1 through week 4 (Figure 10).

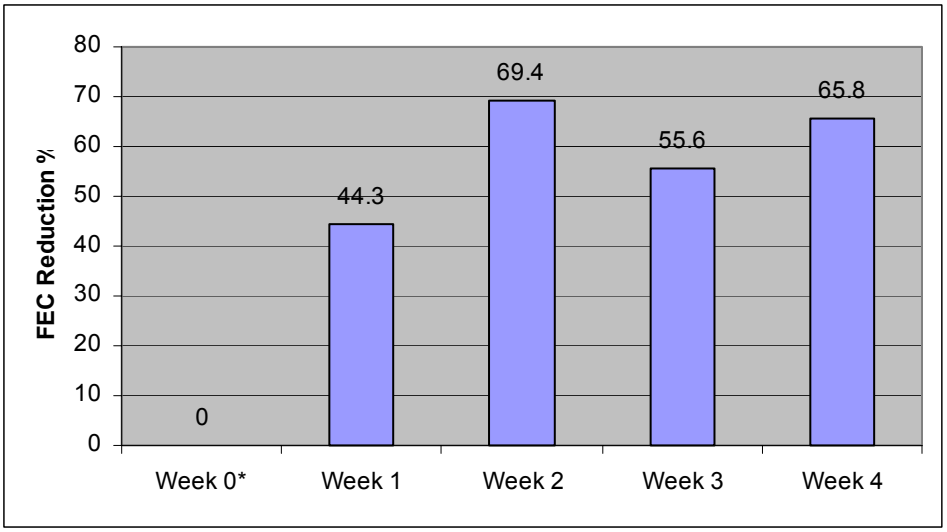

Figure 10. Trial 2 mean percent reduction in FEC comparing copper-oxide wire particle bolus treated and control ewes. *4 gram copper-oxide wire particles bolus administered to the treated group.

4.3.2. Larval Cultures

Trial 2 larval cultures showed that the COWP reduced the population of *H. contortus* at weeks 2 and 4 after administration and this reduction was significant at week 2 (Table 8, Figure 11).

Table 8. Trial 2 *Haemonchus contortus* L3 percentage and percent reduction comparing copper-oxide wire particle bolus treated and control ewes.

Week	0*	2^{+}	4
Control	41.3	50.3	53.3
Treated		23.3	25
Percent reduction	0	53.7	53.1

*4 gram copper-oxide wire particles bolus administered to the treated group. $^{+}P \leq 0.05$

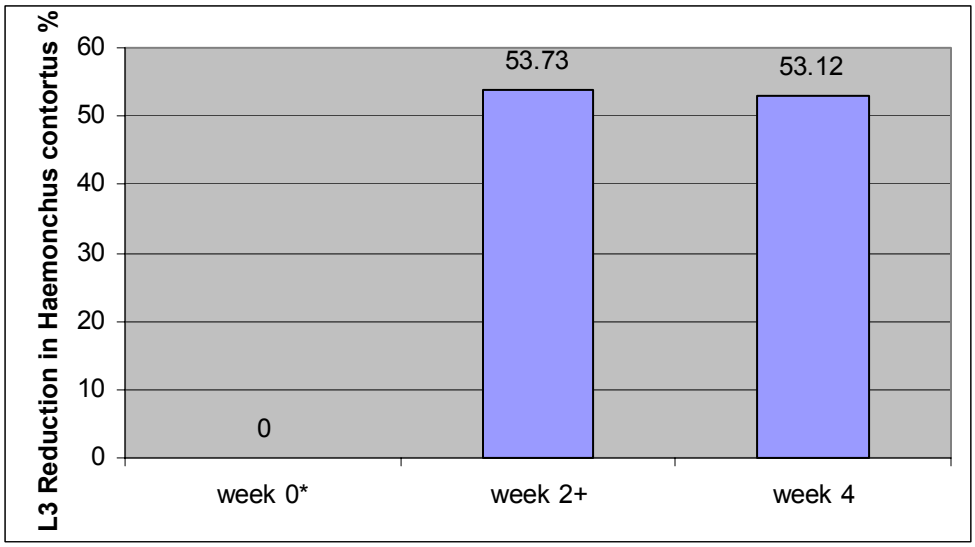

Figure 11. Trial 2 *Haemonchus contortus* L3 percent reduction comparing copper-oxide wire particle bolus treated and control ewes. *4 gram copper-oxide wire particles bolus administered to the treated group. $^{+}P \leq 0.05$

4.4. Trial 3 Ewes

4.4.1. Fecal Egg Count

The FEC was similar between groups at week 0 and significantly reduced from week 1 through week 5 after bolus administration (Table 9, Figure 12).

Table 9. Trial 3 mean fecal egg count and percent reduction comparing copper-oxide wire particle bolus treated and control ewes.

Week	0*	1^{+}	2^{+}	3^{+}	4^{+}	5	6
Control	3910	2667	2080	1520	1490	1317	1423
Treated	3897	553	483	450	620	690	897
FEC reduction (%)	0	79	77	70	58	48	37

*4 gram copper-oxide wire particles bolus administered to the treated group. $^{+}P \leq 0.05$.

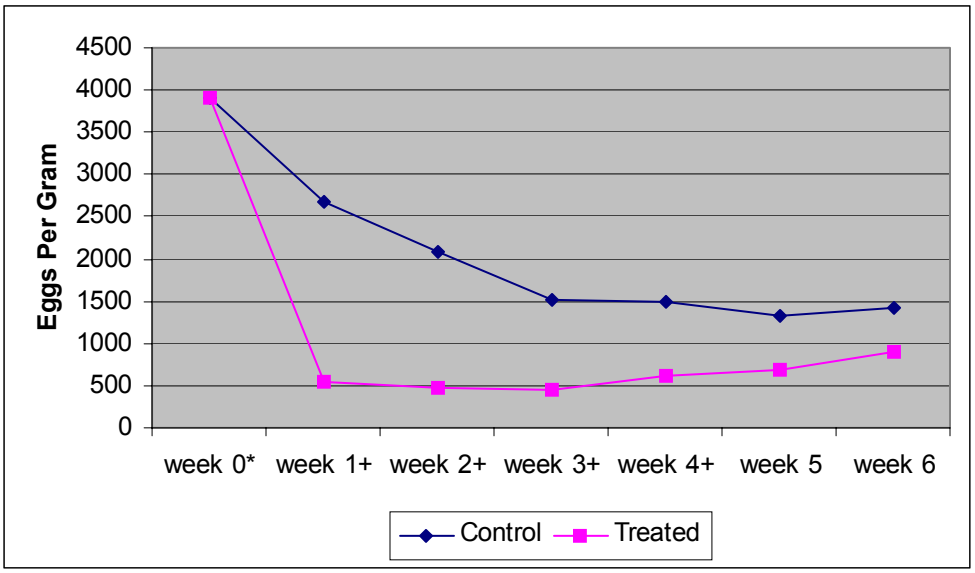

Figure 12. Trial 3 mean fecal egg count comparing copper-oxide wire particle bolus treated and control ewes. *4 gram copper-oxide wire particles bolus administered to the treated group. $^{+}P \leq 0.05$.

Percent reduction in FEC was the greatest at week 1 and continually declined thereafter and reached a low at week 6 (37.0%) (Figure 13).

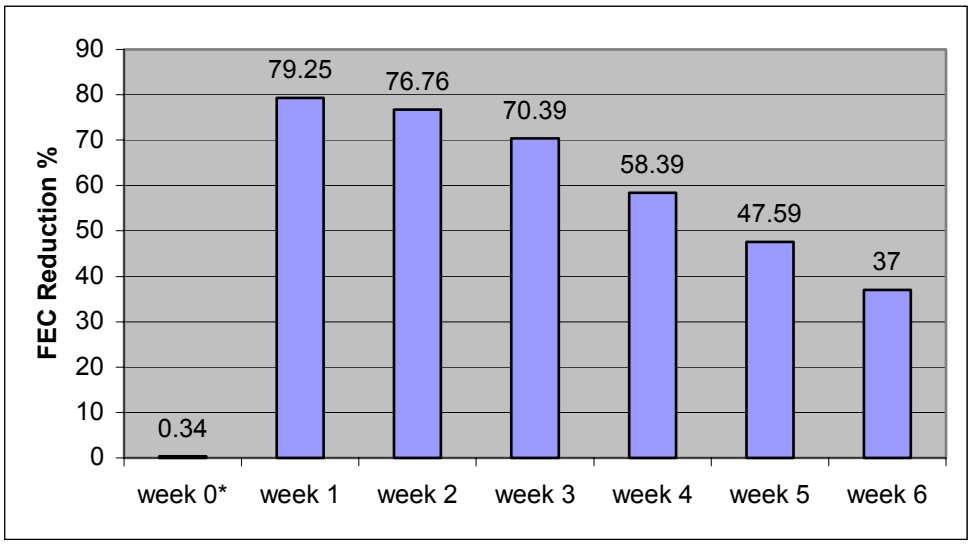

Figure 13. Trial 3 mean percent reduction in FEC comparing copper-oxide wire particle bolus treated and control ewes. *4 gram copper-oxide wire particles bolus administered to the treated group.

4.4.2. Packed Cell Volume

There was essentially no difference in PCV level between groups throughout Trial 3 (Figure 14).

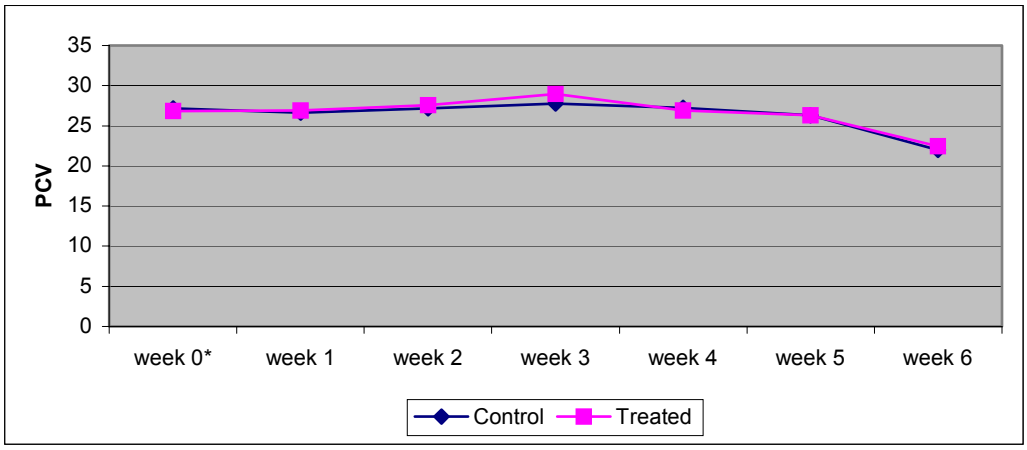

Figure 14. Trial 3 mean packed cell volume comparing copper-oxide wire particle bolus treated and control ewes. *4 gram copper-oxide wire particles bolus administered to the treated group.

4.4.3. Larval Cultures

The larval cultures showed an equivalent level of *H. contortus* at week 0 for both groups (Table 10, Figure 15). Subsequently, the COWP treated group had significantly reduced levels of *H. contortus*, with the highest reduction at week 2 (76.3%) which then continually declined thereafter to a low at week 6 (27.1%).

Table 10. Trial 3 *Haemonchus contortus* L3 percentage and percent reduction comparing copper-oxide wire particle bolus treated and control ewes.

Week	0*	2^{+}	4^{+}	6^{+}
Control	88.6	84.6	76.2	71.1
Treated	86.1	20.1	36.4	51.8
FEC reduction (%)	2.9	76.3	52.2	27.1

*4 gram copper-oxide wire particles bolus administered to the treated group. $^{+}P \leq 0.05$

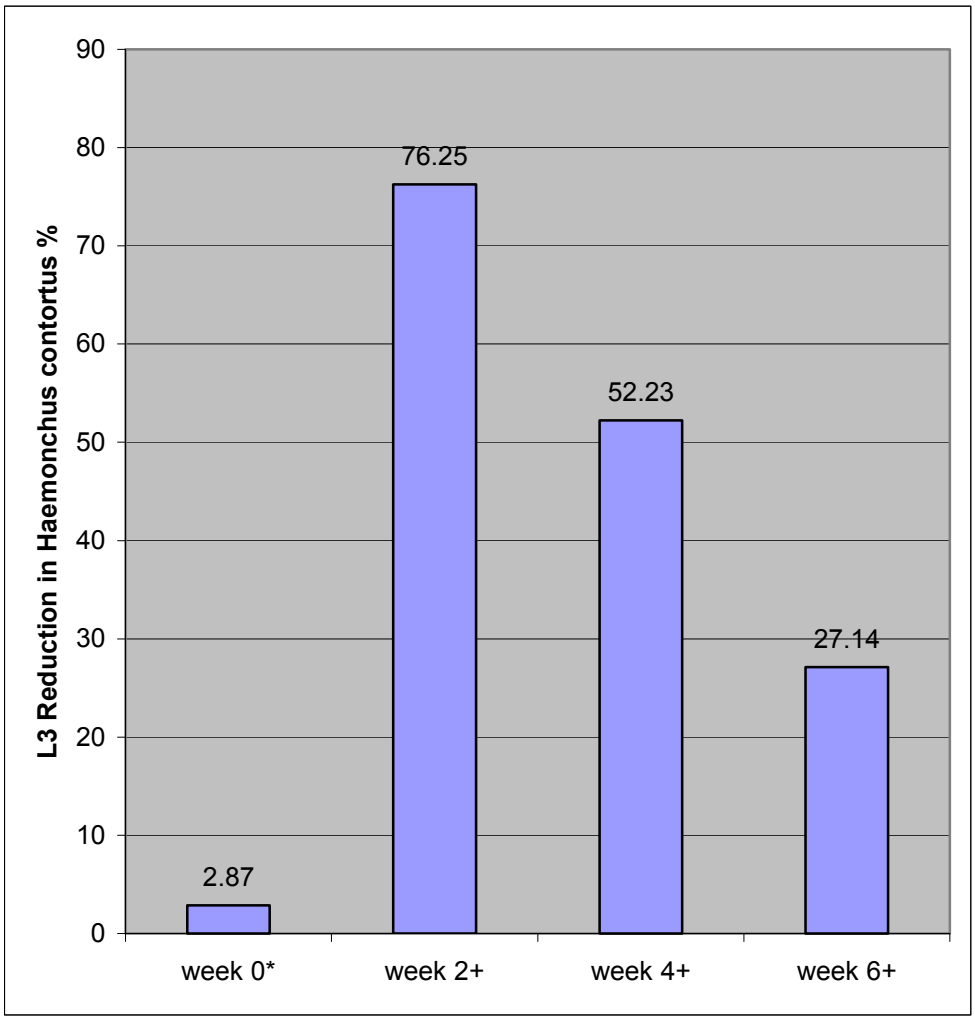

Figure 15. Trial 3 *Haemonchus contortus* L3 percent reduction comparing copper-oxide wire particle bolus treated and control ewes. *4 gram copper-oxide wire particles bolus administered to the treated group. $^{+}P \leq 0.05$

4.4.4. Serum Copper

The serum Cu levels were relatively similar at pre- and post- treatment and were within the normal range of 0.7 – 1.4 ppm (Table 11).

Table 11. Trial 3 pre- and post- treatment serum copper levels per animal in control and treated ewes.

Animal ID	Group	Pre	Post	Difference	Comment
339	Control	1.2	0.9	-0.3	Lower
400	Control	1.1	0.9	-0.2	Lower
607	Control	0.9	1.6	0.7	Higher
1003	Control	0.9	0.9	0	Same
1244	Control	1	1.1	0.1	Higher
1364	Control	1	1	0	Same
1718	Control	0.7	0.7	0	Same
1739	Control	0.8	0.8	0	Same
6659	Control	0.9	1.6	0.7	Higher
6692	Control	0.8	0.9	0.1	Higher
7730	Control	0.8	0.9	0.1	Higher
7793	Control	1	0.7	-0.3	Lower
7938	Control	0.9	0.8	-0.1	Lower
9018	Control	1.1	0.9	-0.2	Lower
9025	Control	0.8	0.8	0	Same
Mean Cu	Control	0.926667	0.966667	0.04	Higher
126	Treated	0.9	1.1	0.2	Higher
307	Treated	1	1.2	0.2	Higher
322	Treated	0.8	0.9	0.1	Higher
335	Treated	0.9	1.2	0.3	Higher
358	Treated	1	0.9	-0.1	Lower
1000	Treated	0.8	0.9	0.1	Higher
1335	Treated	0.8	0.9	0.1	Higher
1368	Treated	0.9	0.7	-0.2	Lower
1746	Treated	0.9	0.6	-0.3	Lower
7828	Treated	1.2	1	-0.2	Lower
7864	Treated	0.8	0.8	0	Same
9024	Treated	0.9	1	0.1	Higher
9247	Treated	0.9	0.8	-0.1	Lower
9290	Treated	0.9	0.8	-0.1	Lower
9538	Treated	1	1	0	Same
Mean Cu	Treated	0.913333	0.92	0.006667	Higher

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1. Discussion

The present study indicated that COWP reduced the infection level of the abomasal nematode *H. contortus*. The activity of COWP was first indicated by a reduction in FEC and was substantiated by larval identification from cultured feces. The activity of the COWP appeared to last for a period of about 4-5 weeks under the conditions for all trials conducted. The percent reduction of *H. contortus* L3 gave an indication of how effective the COWP was at specifically reducing the population of *H. contortus* in the host.

The results seen in Trial 1 ewes presented an unusual observation. After seeing a substantial reduction in week 1, the FEC in the control group all but disappeared in week 2 and the infection didn't return to a level high enough to see any further effect of the COWP. This was believed to be the result of the self-cure phenomenon. By definition, self-cure is the spontaneous expelling of adult worms from the host (Caswell-Chen and Westerdahl, 2002). This occurs when animals are subjected to a very high level of infection over a short period of time. Exsheathment of L3 causes pH changes, and adults are unable to maintain their position. This is suspected to be a hypersensitivity reaction that results in expelling of adult worms.

In other studies, reduction of *H. contortus* wasn't measured by larval reduction in fecal cultures, but by parasite recovery at necropsy and FEC (Bang et al., 1990a; Knox, 2002; Chartier et al., 2000). Reduction in *H. contortus* adults ranged from 37% to greater than 95% with an average percent reduction of 66%. Percent reduction in FEC was

higher, as seen by Chartier et al. (2000) and Knox (2002) whose egg output was lowered by 37-95% and >85%, respectively.

Although previous results showed the effectiveness of COWP, the methods used were different from those used in the three trials of this study. These differences were 1) amount of COWP used – 5g (Bang et al., 1990a); 2) experimental infection with *H. contortus* (Bang et al., 1990a; Knox, 2002; Chartier et al., 2000); and 3) the method in which *H. contorus* reduction was measured – parasite recovery (Bang et al., 1990a; Knox, 2002; Chartier et al., 2000) and FEC (Knox, 2002; Chartier et al., 2000).

The highest percent reduction (96%), by way of parasite recovery was seen by Bang et al. (1990a) and the highest percent reduction in FEC (95%) was seen by Chartier et al. (2000). The results in this experiment cannot be directly compared to those studies, but the trend in effective reduction is similar with the highest percent reduction of *H. contortus* L3 (78%) and FEC (99%) in Trial 1 lambs.

Since this is a new approach to nematode parasite control, there is little information available addressing the mechanism(s) behind how the COWP affects *H. contortus* and/or other abomasal nematodes.

There are several possible mechanisms that can be postulated from the constant release of soluable copper from the COWP in the abomasums. One such mechanism could involve alteration of the reproductive capability of *H. contortus* in the host. The lower FEC and percentage of L3 in the COWP treated animals could be explained by a reduction in the prolific egg-laying ability of *H. contortus*. Reducing the reproductive capabilities of *H. contortus* will result in decreased egg output. This lowered egg output, decreases the percentage of larvae on pasture available to infect the grazing animals and;

therefore, the overall free-living population is reduced. Infection levels in the grazing host would consequently be reduced. In addition, the residual copper in the abomasum could be passed out in the feces and somehow interfere with larval survival and development, thus leading to fewer L3 to be picked up by grazing animals.

The high concentration of copper in the abomasum (an acidic environment whose normal solubility of copper is much lower than other regions in the digestive tract, Mills, 2003) may also reduce the pathogenic potential of *H. contortus*. Copper is an important component for maintaining immunocompetence. If an animal is relatively copper deficient, it may be more susceptible to *H. contortus* infection. In the presence of increased copper levels the ability to mount an enhanced immune response could result in the expelling of *H. contortus* L3s, L4s, and adults from the host. For example, Howell and Gawthorne (1987) observed that lambs from rams selected for low plasma copper concentrations were found to be twice as susceptible to a variety of infections as those from rams selected for high plasma copper concentrations. In another example, a highly significant reduction in the number of antibody producing cells in animals fed low dietary copper was observed (Newberne et al, 1968). Although these two examples don't directly measure the effect COWP has on *H. contortus* in the abomasum, a probable correlation can be seen.

Another possible mechanism that could result in the reduced FEC and *H. contortus* infection levels involves the environment of the abomasum. Due to the high concentration of soluble copper, the pH of the abomasum is lowered. This low pH could make the environment in the abomasum unsuitable for *H. contortus*. Bremner (1961) has suggested that soluble copper can penetrate the cuticle of helminths. This could affect

the nematodes mobility, ability to feed, and overall functions and, thus, lead to expulsion and/or death.

The actual mechanism of *H.* contortus blood feeding involves attachment to the abomasal mucosa and extrusion of its' oral lancet to slit capillaries. They ingest blood flowing from these slit capillaries. They also secrete an anticoagulant into the bleeding lesion ensuring that these lesions will continue to bleed after the worm is satiated and has moved away (Johnstone, 2003). It is possible that during ingestion of blood other abomasal fluids containing high levels of copper are taken in and thus affect in some way the life processes of the nematode from within which may lead to immobilization and/or death.

These are all possibilities, and further research needs to be done, so the effect of COWP on nematodes can be fully understood and appreciated.

5.2. Conclusion

Treatment with COWP reduced the establishment and/or fecundity of *H. contortus* for an extended period. The use of COWP in conjunction with other control methods may be a very useful tool for producers and help reduce reliance on the conventional use of anthelmintics for control.

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VITA

Ariane Diane Watkins was born in New Orleans, Louisiana, in 1978 to Mr. And Mrs. Adrian Watkins, Sr.. She has three other siblings and is the youngest child to her father. She gained her secondary education by attending the public schools in Orleans parish. After graduating in the top of her class from McDonogh # 35 Senior High School in 1996, she attended Louisiana State University in Baton Rouge, Louisiana. After five years, she completed her Bachelor of Science degree in animal science, May, 2001. She began work on her master's degree (major - veterinary medical sciences) at Louisiana State University, Fall of 2001 in the Department of Pathobiological Sciences. She will graduate in August 2003.